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Technical Report

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Health Risk Assessment of Women in Submarines: Reproductive and Developmental Toxicity Evaluation of Major Submarine Atmosphere Components (CO, CO_2, and O_2) in Rats (*Rattus norvegicus*) – Phase I (Range Finding Study)

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Abstract

Recent congressional approval allowing women to serve in the submarine service necessitates additional investigation into the suitability of existing submarine breathing air standards for women. This study evaluates the general, reproductive and developmental health effects upon male and female rats exposed to mixtures of three critical submarine atmospheric components (CO, CO_2, and O_2) at concentrations that represent the current submarine standards for normal operating conditions (90 days), 24-hour short-term exposure limits, and 1-hour emergency exposure limits. The study is divided into three consecutive phases designed to determine whether the existing standards for these gases are health protective of male and female submarine crew members. This first phase is a short term range finding study to rapidly screen for overt toxicities after 14 days of exposure, with a focus on reproductive physiology in male and female rats. The second phase will provide a 28-day exposure evaluation of neurological and reproductive performance. The third phase will provide a 90-day sub-chronic exposure, two generation developmental and reproductive toxicity (DART) study, which will also evaluate the reproductive ability of offspring not exposed *ex utero*. This technical report presents the findings of the first phase of the study, which evaluated four groups of 16 male and 16 female rats exposed via whole body inhalation to clean air (0.4 ppm CO, 0.1% CO_2, 20.6% O_2), a low-dose gas mixture (4.6 ppm CO, 0.4% CO_2, 17.1% O_2), a mid-dose gas mixture (13.9 ppm CO, 1.2% CO_2, 16.1% O_2) and a high-dose gas mixture (88.4 ppm CO, 2.5% CO_2, 15.0% O_2) for 23 hours per day for 14 consecutive days. The exposure concentrations were well tolerated by the rats and will be used in the subsequent phases. Pathological findings were unremarkable, or

incidental to exposure, and blood analysis results were within normal clinical parameters. There were no identified effects to reproductive tissues from exposure to the mixtures. Due to the short duration of the exposures in this range-finding phase of the study, no definitive conclusions should be drawn at this time regarding the toxicities, or lack thereof, of these mixtures.

Keywords: Inhalation, carbon monoxide, carbon dioxide, hypoxia, reproductive toxicity

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Introduction

Submarine atmospheres present a unique and closed occupational environment, with personnel being exposed to low-level concentrations of chemicals and chemical mixtures for 24 hours per day on a sub-chronic (≥ 90 days) basis. Whereas Congress has recently passed legislation that allows women to serve aboard submarines, it is imperative to re-evaluate the current submarine breathing air standards, such as emergency exposure levels (EELs) and continuous exposure levels (CELs), with a special focus upon potential reproductive and developmental effects, as well as gender-specific effects. Based on previous efforts (National Research Council 2007, 2008, 2009), the atmospheric components of carbon monoxide (CO), carbon dioxide (CO_2), and oxygen (O_2) are considered among the highest concerns in submarine atmospheres. While there is limited data on these individual components with regard to reproductive and developmental effects, to our knowledge there are no data assessing the various combinations of these gases as a mixture, nor for assessing the adverse health effects of these gases after prolonged, continuous (24 hour per day) exposures.

Assessing the health risk to female crew members in submarines is a complex and controversial issue (Kane and Horn, 2001). This research is being conducted to better elucidate the potential impacts of these mixed gases upon male and female reproductive and developmental health, as well as the overall mission effectiveness of the submarine community. When adequate human data are lacking, the primary alternate method for establishing the health risk from a chemical substance is to perform toxicity studies in animals, and then use the research principles that have been proven to be predictive, robust and valid for extrapolating the animal results to humans.

The purpose of this study is to evaluate the general, reproductive and developmental toxicity within male and female rats exposed via whole body inhalation methods to key combinations of the three most critical submarine atmospheric components (increased CO, increased CO_2, and decreased O_2), and to use these data to guide the subsequent phases of this study.

The O_2, CO and CO_2 exposure concentrations were selected based upon existing standard limits promulgated within the *Technical Manual for Nuclear Powered Submarine Atmosphere Control* (NAVSEA S9510-AB-ATM-010 REV 2). Low-dose group concentrations are based upon the 90-day CEL (average onboard levels); mid-dose group concentrations are based upon the 24-hour CEL (maximum onboard levels); and, high-dose group concentrations are based upon the 1-hour EEL (emergency levels).

Four groups of animals (target concentrations) were exposed to clean air (0.4 ppm CO, 0.1% CO_2, 20.6% O_2), a low-dose (4.6 ppm CO, 0.4% CO_2, 17.1% O_2), a mid-dose (13.9 ppm CO, 1.2% CO_2, 16.1% O_2) and a high-dose (88.4 ppm CO, 2.5% CO_2, 15.0% O_2) for 23 hours per day for a period of 14 consecutive days. Each exposure group was stagger-started by one day.

Experimental Design

The study will be performed in three consecutive phases. Phase 1 (range finding) is described in this report and involved continuous exposures to male and female rats for 14 days to relevant test atmospheres with toxicity assessments performed on vital organs and reproductive tissues. Phase 2 of the study will expose male and female rats to the same atmospheres for 28 days, with neurological and reproductive performance assessed, in addition to general toxicity. The first generation rats (F1) from Phase 2 will also be assessed for general health conditions and gross malformations, but will not be exposed to the test atmospheres. Phase 3 (sub-chronic) of this study will be modeled after the USEPA guidelines for assessing "*Reproduction and Fertility*

Effects" (OPPTS 870.3800). The male and female rats will be exposed to the same three test atmospheres for a continuous 90 day period that includes gestation, and will be assessed for toxicity, as well as neurological and reproductive effects. F1 offspring will not be exposed *ex utero*, but will be evaluated for general toxicity and malformations, as well as neurological and reproductive abilities. F2 offspring resulting from the mating of F1 rats will not be exposed, but will be evaluated for toxicity and gross malformations to assess any delayed developmental effects or toxicity, and to assess the reproductive ability of the F1 generation.

Materials and Methods

Animal Protocol (F-WA-2010-0116-A)

A total of 128 CD® IGS rats, 51-54 days-old, were purchased from Charles River Laboratories (Wilmington, MA). The rats were randomly divided into four groups of 16 males and 16 females. The rats were provided husbandry conditions consistent with practices recommended by the Association for Assessment and Accreditation of Laboratory Animal Care (AAALAC), and in compliance with the National Research Council's "Guide for the Care and Use of Laboratory Animals" (ISBN-10:0-309-15400-6). The rats were provided a two week quarantine period in the animal vivarium, which included four days of acclimatization to exposure cage units (cage training). Cage training involved placing the rats in the stainless steel cage units for increasing periods of time (2, 4, 6, 8 hours) on four different days during the week prior to the study start, and returning them to polycarbonate cages between training periods. Following acclimation, the rats were placed in the cage units for the duration of the inhalation study except when the cages were changed (weekly), animals were weighed (weekly) and female animals were monitored for potential estrous cycle alterations via vaginal lavage and cytology assessment. Food and water were available to all animals *ad libitum* throughout the experiment, and all rats were kept on a 12 hour light/dark cycle during exposure.

Chemicals

Rats were exposed to clean air or mixed atmospheres of carbon monoxide, carbon dioxide or oxygen. Clean air for the control and exposure system was from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake to replace used air through a high-efficiency particulate air filter (HEPA). One cylinder (4.25 cubic meters) of CO (99.999%) and fifteen cylinders (22.65 cubic meters per cylinder) of CO_2 (99.995%) were purchased from Weiler Welding, Dayton, OH. The O_2 concentrations were reduced to test conditions by dilution with the appropriate amounts of nitrogen (N_2) provided from a nitrogen generator (Parker Balston Model DB-5, Summit Industries, Inc., Dayton, OH). The nitrogen generator produced 95 to 99% N_2 from in-house compressed air filtered for water and oils.

Inhalation Exposure Chambers

Rats were exposed in a one cubic meter whole body exposure chamber (1 m^3, H1000, Lab Products, Seaford, DE). One chamber was used for each concentration including a control chamber. Stainless steel caging (R-32, Lab Products, Seaford, DE) was used to contain the animals during inhalation exposures and served as the domiciliary housing during periods of non-exposure. Each R-32 cage unit housed 32 rats, and was placed in the middle section of each 1 m^3 chamber. The dimensions for each rat compartment within the R-32 cage units were 14.0 x 14.5 x 20.3 centimeters (W x L x H) and provided 203 square centimeters of floor space. Stainless steel pans were placed under each set of stainless steel cages to collect the urine and feces. Urine and feces pans were changed daily for the duration of the inhalation exposures.

Inhalation Exposure Chamber Operation

The inhalation exposure chambers were operated as a push-pull system. Air was pushed into the inlet of the chambers from an air circulating system using a turbine blower (The Spencer Turbine Co., Windsor, CT) with a room air intake to replace used air through a high-efficiency particulate air (HEPA) filter and air was pulled from the exhaust of the chambers through a manifold using an exhaust fan on the roof on the facility.

The target inlet air flow rate in the mixed atmosphere chambers was set to 200 to 250 L/min, providing approximately 12 to 15 air changes per hour. Inlet air flows were a sum total of the clean air, CO flow, CO_2 flow and N_2 flow. Inlet air flows were controlled by a manually operated gate valve. Inlet air flows were monitored by mass flow monitor (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA) connected to a laminar flow element (Model HFM-200 LFE, Teledyne-Hastings Instruments, Pittsburgh, PA). Each of the mass flow monitors were connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh, PA).

The inlet air flow for the control chamber was initially set to the target range of 200 to 250 L/min, but was increased to approximately 390 L/min to dilute the CO_2 concentrations produced by the exhaled breath of the animal load. The higher flow rate to the control chamber resulted in somewhat lower humidity conditions for rats in the control group in comparison to the rats from the dose groups, but this difference is not expected to affect study results.

The chamber exhaust flow for the mixed atmosphere exposure chambers was adjusted with a manually operated gate valve to maintain a slight negative pressure relative to the room during the exposure to prevent the test atmosphere from entering the laboratory area in the event of leaks. The control chamber exhaust flow was adjusted to maintain a slight positive pressure relative to the room on Day 1, but was increased to approximately 390 L/min to dilute the CO_2

produced by the exhaled breath of the animal load. The control chamber door latches were left open to allow room air to enter thereby diluting the CO_2 to the lowest concentrations possible.

The static pressure of each inhalation chamber was determined using both a magnahelic gage (Model 2304, Dwyer Instrument Co., Michigan City, IN) with a large visual display and electronic sensor (Model ZPS-05-SR09-EZ-ST-D, Building Automation Products, Inc., Gays Mills, WI).

Temperature and Humidity

Temperature and relative humidity were measured by a temperature and relative humidity probe (Model HF532WB6XD1XX, Model HC2-S, Rotronics Instruments, Inc., Hauppauge, NY) located inside of each exposure chamber. The target temperature was maintained between 18 – 26 °C and the target relative humidity was between 30 and 70%.

Atmosphere Generation

All test chemical gases for the mixed atmospheres were metered by mass flow meters (Model HFC-202, Teledyne-Hastings Instruments, Pittsburgh, PA) at flow rates appropriate to maintain target concentrations of mixed atmospheres for each of the target doses. Each of the mass flow meters were connected to a four-channel power supply (Model THPS-400-115, Teledyne-Hastings Instruments, Pittsburgh PA) and manually adjusted to the appropriate channel of a four channel power supply. Figure 1 shows a diagrammatic representation of the exposure system.

Test Atmosphere Monitoring

The mixed test atmosphere of each of the four inhalation chambers was monitored continuously with a multiple gas analyzer (Model VA-3113, Horiba Instruments, Inc. Moon Township, PA). Each instrument contained a magnetopneumatic (MP) sensor for O_2 measurements and two non-dispersive infrared analyzers (NDIR) for CO and CO_2 measurements. Each instrument was

calibrated using a N_2 dilution manifold and varying amounts of calibration gases (Airgas, Dayton OH): 500 ppm CO in N_2, for the CO NDIR, 5% CO_2 in N_2 for the CO_2 NDIR, and room air (20.9% O_2) for the O_2 MP. Each instrument was zeroed using N_2.

Automated Alarm System

The monitoring sensors for the key parameters of temperature, relative humidity, airflow, CO concentration, CO_2 concentration and O_2 concentration within the inhalation chambers were electronically connected to an alarm system (Model FGD-2000, Sensaphones, Phonetics, Inc., Aston, PA) that automatically contacted the subject matter expert (SME) if the electronic signal fell outside of the acceptable range, ensuring prompt correction.

Exposure Data Collection

Data were collected via automation by a computer using data collection software (LabView Software v.10.0, National Instruments, Austin, TX). Data were collected every 10 seconds for temperature, humidity, supply air flow, CO concentration, CO_2 concentration, O_2 concentration and static pressure for all groups. In addition, for the low, mid and high dose groups, data were also collected every 10 seconds for CO flow rate, CO_2 flow rate and N_2 flow rate. The 24-hour daily data for each dose group were collected from 0900 until 0900 the following day. Periods when the chambers were opened for animal husbandry and animal procedures were included in the daily averages to reflect the actual average exposure concentrations experienced by the rats. At the end of each day, the average, standard deviation, minimum values, maximum value and the total number of data values were calculated. Daily averages were used to calculate the average of daily averages, standard deviation of daily averages, minimum daily average, maximum daily average and number of daily averages.

Study Day

A study day was defined as a 24-hour period generally from approximately 0900 until 0900 the following day. The study days were numbered consecutively from 1 to 18 corresponding to the first day when the control group was loaded into the control chamber until the last day when the final exposure group was removed from the high-dose chamber and reflected the staggered schedule for initiating exposures for the four groups. Exposures were interrupted daily for 15-60 minutes for removal of urine and feces; inspection and changing of equipment; and, observation of rats for health and well being, and weight and estrous cycle phase changes.

Necropsy

On the day of the necropsy, male and female animals were anesthetized until unresponsive by CO_2 overdose, and then blood was sampled via cardiac puncture. After blood collection, the rats were decapitated and all target organs were harvested for analysis. Blood/serum was collected and processed for clinical chemistry and hematology analyses following standard laboratory procedures. Target tissues were harvested using standard necropsy methods. All blood and organ tissue samples were frozen at - 80^{o}C until processed for analysis.

Estrous Cycle

Estrous cycle phases were categorized for all female rats (16 per group) by employing vaginal lavage methods previously published by Marcondes, *et al.* 2002. Dose group comparisons to controls were based on the proportion of observed rat-days within each of the estrous cycle phases during a five day cycle. The metestrus and diestrus phases were combined into a single category. The evaluation period began following a full estrous cycle under exposure conditions. If the categorization was ambiguous (e.g., designated as positive for both the proestrus and the estrus phases), then each phase category was scored as an observation of 0.5 rat-days. If an insufficient number of cells were recovered to categorize an estrous cycle phase, then the data were excluded; as a result, the total number of rat-days sometimes varied between groups. The

proportional differences between the dose groups and the controls were evaluated for statistical significance ($\alpha = 0.05$) for each of the estrous cycle phases.

Hematology

Complete blood count (CBC) analysis was performed on 40 µL samples of whole blood taken from each animal using a HemaVet® HV950 Blood Analyzer (Drew Scientific, Inc., Waterbury, CT). Parameters measured were: number per µL of white blood cells (WBC), red blood cells (RBC), lymphocytes (LY), monocytes (MO), and granulocytes (neutrophils (NE); eosinophils (EO); basophils (BA)), % LY, % MO, % NE, % EO, % BA, grams hemoglobin (HB) per dL, % hematocrit (HCT), mean corpuscle volume (MCV), mean corpuscular hemoglobin (MCH) per pg, mean corpuscular hemoglobin concentration (MCHC) per dL, red blood cell distribution width (RDW), and number per µL of platelets (PLT).

Serum Chemistry

Serum chemistries were measured using a VetTest® 8008 Chemistry Analyzer (IDEXX Labs, Inc., Westbrooke, ME) and VetLyte® Electrolyte Analyzer (IDEXX Labs, Inc., Westbrooke, ME). A 100 µL sample of serum from each animal was analyzed for total protein (TP), albumin (ALB), alkaline phosphatase (ALKP), alanine aminotransferase (ALT), aspartate aminotransferase (AST), blood urea nitrogen (BUN), cholesterol (CHOL), creatinine kinase (CK), creatinine (CREA), globulin (GLOB), glucose (GLU), total bilirubin (TBIL), triglycerides (TRIG), and major electrolyte concentrations (Na^+; K^+; Cl^-).

Tissue Histopathology

Formalin fixed, paraffin embedded, 5 micron, hematoxylin and eosin stained sections of select tissues were submitted to LTC Deidre Stoffregen (VC, USA), 711th Human Performance Wing

(HPW/RHP), WPAFB, OH, for histopathological analysis. The following tissues were prepared for evaluation: brain (basal ganglia, hippocampus and hypothalamus), heart, pancreas, liver, spleen, kidneys, adrenal glands, pituitary gland, male reproductive organs (testes, seminal vesicles and prostate), and female reproductive organs (ovaries, uterus, cervix and vagina).

Statistics

The two-proportion, two-tailed, z-test was used to analyze the difference between proportions at the 0.05 significance level (α=0.05). Data analyzed by the z-test included: (1) the proportion of time spent in each estrous cycle phase for each dose group in comparison to controls; and, (2) the proportion of rats exhibiting specific lesions for each dose group in comparison to controls. All proportions were based upon incidence data only, and not the severity of a histologic lesion. Quantitative data were expressed as a standard deviation of the mean and statistical analyses were performed using SYSTAT 13® (Systat Software, Inc., Chicago, IL). Two-way analysis of variance (ANOVA) was used to compare changes in weight, hematology and serum chemistry values between groups differentiated by both exposure and gender. Changes were assessed for significance at $P \leq 0.05$. When no significant difference was identified based upon gender, male and female groups were combined to increase the power of the test to determine the changes based upon exposure. Dose groups with significant effects were identified using post hoc Tukey–Kramer procedures for multiple comparisons.

In severe cases of observed blood clotting or hemolysis (≤ 5% of data), which can frequently result in anomalous hematological and/or serum chemistry values, anomalous data were only discarded from the statistical analysis after passing the Grubbs test for identifying outliers.

Results and Discussion

Environmental Parameters

The whole body inhalation exposure system, which was developed specifically for this project, performed very well and proved the laboratory's capability to control test conditions within the parameters specified by the study protocol. The lone exception was the lower humidity in the control chamber as discussed above in Materials and Methods. The performance data for environmental parameters are provided in Table 1. The data for the test chemical flow rates are provided in Table 2. Figure 1 shows a diagrammatic representation of the exposure system. Based on these results, no alterations are deemed necessary for the subsequent phases of this study.

Deaths

No animal deaths occurred within the control or dose groups during the 14-day exposure period in phase 1 of this study. Additionally, no animals were removed from their respective exposure test atmospheres beyond the 1-hour daily limit allowed for routine husbandry and performing the vaginal lavage, as described previously.

Body Weights/Body Weight Gains

Differences in animal weights were compared with repeated measures ANOVA. Dose group and gender were between group factors and days on study was the within subject factor. The U.S. EPA Benchmark Dose Technical Guidance Document (EPA/630/5-00/001, 2000) was used to determine the significance of any weight difference found between treatment and control groups with ≤ 10% considered insignificant. For female rats, the average weight gain over the 14 day exposure period for all three dose groups was not significantly different from controls. However, weights of male rats in the dose groups were significantly higher compared to controls [$F(3, 123) = 5.05$, $p \leq 0.002$] from the initiation of the study. Male rats in the mid- and high-dose groups were 13 - 33 grams heavier than controls at the initiation of exposures. By comparison, the average weight of females across the dose groups varied by only 3-5 grams, suggesting

that the females were more uniform in maturity than were the males at the beginning of the exposure period. The reason for the high variation among the males is unknown considering that all rats were randomly selected to dose groups. Significant differences were also noted for body weight gains between the dose group males, compared to controls [$F(2, 246) = 201.34$, $p \leq 0.001$].

Estrous Cycles

There were no indications that exposures to the various test gas mixtures had any effect upon the estrous cycle. The proportions of the exposed animals which were observed in the various estrous cycle phases were not statistically different from estrous cycle phase categorization of the control animals. Results are summarized in Table 3. Monitoring of the estrous cycle will continue in subsequent phases of the study.

Tissue Weights

Mean tissue weights are reported in Table 4 (females) and Table 5 (males). The mean weight of male sex organs taken from rats in the mid-dose group was statistically significantly lower ($p \leq 0.05$) in comparison with controls. However, as this difference was not observed in the high-dose group this result is considered not treatment related. No other organs examined were found to have statistically significant differences in their weight.

Hematology Values

Hematology results from blood drawn during necropsy are reported in Table 6 (females) and Table 7 (males). A series of two-way ANOVAs were conducted to test for statistical differences in hematology values based on exposure and gender, followed by Tukey–Kramer procedures for multiple comparisons. No statistically significant gender differences were identified. Two significant differences based on exposure concentrations were identified for both males and females, including: (1) increased red blood cell distribution width (RDW) in rats from the high-

dose group, in comparison with all other groups [F (3, 71) = 5.61, $p \leq 0.002$]; and, (2) decreased platelet concentrations in rats from the control group in comparison to the other three dose groups [F (3, 71) = 12.56, $p \leq 0.001$]. While the differences in platelet counts are statistically significant, they are not considered clinically relevant as they are not outside the normal ranges for these animals (Tables 8 and 9). Moreover, the finding of decreased platelet concentrations may be attributed to higher than normal blood clotting and hemolysis observed in several specimens in the control and the mid-dose groups. Previous studies (Lewis, *et al.,* 1985) have shown that hemolysis can affect platelet counts; hence, it is uncertain whether this finding indicates a dose related response or anomalous results in the control group's analysis. Subsequent phases of this study will incorporate new phlebotomy procedures that will reduce the potential for clotting and hemolysis in the blood.

The increase of RDW in the high dose group is indicative of increased erythropoiesis. This finding is consistent with a normal physiological response to hypoxia, in this instance induced by the low concentration of O_2 and the elevated concentrations of CO and CO_2 used for the high-dose group over the 14 day exposure period. Non-statistically significant increases were observed in the RBC counts and hemoglobin concentrations for the high-dose group as well (Tables 6 and 7), and are also consistent with hypoxic conditions and a normal physiological response. As phase 1 was conducted over only a 14 day period, it will be informative to note if these findings remain the same, are more pronounced, or are resolved as exposure durations increase during phase 2 and phase 3 of this study.

Serum Chemistry

Serum chemistry results from blood that was drawn during necropsy are reported in Table 8 (females) and Table 9 (males). A series of two-way ANOVAs were conducted to test for any statistical differences in serum chemistry values based on exposure and gender, followed by

Tukey–Kramer procedures for multiple comparisons. When the data were assessed for statistical significance, the only gender dependent difference identified was an increase of albumin concentrations in male rats from the mid- and high-dose groups compared to controls [$F(3, 55) = 3.58$, $p \leq 0.019$]. Four significant changes were identified for males and females based on exposure, including: (1) an increase in the potassium ion concentrations in rats from all three dose groups compared to controls [$F(3, 107) = 6.605$, $p \leq 0.001$]; (2) an increase in glucose concentrations in rats from the high-dose group compared to rats from all other groups [$F(3, 110) = 7.71$, $p \leq 0.001$]; (3) an increase in creatinine concentrations in rats from the high-dose group compared to controls [$F(3, 112) = 4.58$, $p \leq 0.005$]; and, (4) an increase in albumin concentrations in rats from the high-dose group compared to controls [$F(3, 111) = 3.94$, $p \leq 0.010$]. While the differences described above are statistically significant, over interpretation should be avoided at this time as all mean values for these parameters fell within the normal ranges established for these animals. The blood chemistry results presented in this section will be further discussed in the context of the histopathology findings (see the next section).

Histopathology

Tissues from control and dose groups were prepared following necropsy for microscopic examination. The tissue findings identified through histopathology are summarized in Table 10 (females) and Table 11 (males). No significant lesions were identified in the adrenal glands, brain (basal ganglia; hippocampus; and hypothalamus) or female reproductive organs (ovaries; uterus; cervix; and vagina). All of the female reproductive organs appeared to be normal and exhibited the normal features for the respective estrus cycle phase at the time of necropsy. Idiopathic lesions were observed in the heart, pituitary gland, spleen, epididymis and seminiferous tubules; however, the data indicate that these lesions were incidental to the mixed gas exposures due to the fact that all other evaluated sections of these tissues were normal.

A statistically significant increase ($p \leq 0.05$) in the incidence of pancreatic acinar cell atrophy in comparison to controls was found for the mid- and high-dose groups in male rats (Table 11) and for the high-dose group in female rats (Table 10). Pancreatic atrophy is a spontaneous degenerative change that occurs in young adult rats with a 3-6% incidence rate (Chiu, 1983). As phase 1 used a relatively short exposure duration of 14 days, definitive identification of a dose-response for this finding will require confirmation from phase 2 (28 day) and phase 3 (90 day) of this study.

Small clusters of infiltrates (inflammatory cells) were commonly observed within the hepatic sinusoids of the liver across all groups, including controls. Although the background incidence of this minimal finding is naturally high for this particular strain of rat, the statistical increase in the proportion affected in comparison to controls ($p \leq 0.05$) was significant for males in all dose groups, and for females in the mid- and high-dose groups. As described above for the pancreatic findings, the liver findings will need to be confirmed in the subsequent phases of this work before their relevance can be properly assessed.

A statistically significant increase ($p \leq 0.05$) in the incidence of prostatitis, a chronic interstitial inflammation of the prostate gland, was observed in the males from the high-dose group, in comparison to controls. A high incidence of spontaneous prostatitis has been found in other studies (Keith et al, 2001). Additionally, there was no evidence of degeneration or atrophy of the accessory sex glands; therefore, these observations are not considered clinically relevant and may be incidental to exposure.

An observation of early stage chronic progressive nephropathy, another degenerative disease commonly found in this strain of rat (Turnbull, *et al*. 1985), was found in 78% of animals examined across all groups. The cause of the disease is multi-factorial, but protein overload is

the most common cause, with the proximal convoluted tubule being the most common site for this lesion. The clinical severity of the lesions was minimal, and there was no evidence of ischemia or hypoxia present in examined sections; therefore, these findings are considered incidental at this time. No statistically significant difference was found in mean percentage incidence of renal tubular degeneration between the dose groups and the controls. The progressive nephropathy finding from phase 1 will continue to be evaluated in conjunction with the findings from the subsequent phases.

When considering the hematology, chemistry and histopathology data together, several findings are noteworthy. First, exposure to high concentrations of the gas mixtures resulted in mild elevations of blood glucose and potassium concentrations. These serum chemistry changes may be associated with the histopathological alterations observed in the pancreas and liver, but the results from longer term exposures will be required to verify if these observations are dose-related or incidental. Second, the mid and high dose groups showed an increase in albumin and creatinine, with hematocrit at the upper end of its normal range, which collectively may be indicative of mild dehydration. Since the rats were given constant access to water, an alternative explanation may be a mild acidosis from respiring high CO_2 concentrations. Prolonged dehydration is also a potential contributing factor of nephropathy. No definitive statements can be made at this time as to the relevance of these findings. The results from the longer term exposures will be required to put these present findings into the proper context.

Conclusions

The purpose of phase 1 of this study was to observe whether the exposure of rats to the specified test atmospheres for 14 days would produce gross physiological changes or indicate a need for alterations to subsequent phases of the study. The results of this 14-day range-finding

study indicate that the observable health of rats from the dose groups was clinically similar to controls, and that there is no need to alter the test mixtures for subsequent phases of the study.

Although early indications of clinical disease (chronic progressive nephropathy) were identified in the dose groups, similar endpoints were discovered in the control group, and were not found to be statistically significant in comparison to controls. These observations are common among aging rats of this species, particularly male rats. With regard to the other findings of note (histopathology, chemistry and hematology) as described in the results section, definitive interpretations of these findings cannot be properly assessed until the results from the longer duration exposures (phase 2 and phase 3) are completed and it would be premature to speculate further on their meaning at this stage of the study.

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Table 1: Inhalation exposure summary data: environmental parameters

			Group 1 Clean Air Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Temp	(Deg C)	**Mean**	**21.2**	**22.7**	**22.8**	**22.7**
		St Dev	0.3	0.4	0.3	0.2
		Min	20.7	22.3	22.3	22.5
		Max	22.1	24.0	23.6	23.0
		Count	15	15	15	15
Humidity	(%)	**Mean**	**21**	**37**	**35**	**40**
		St Dev	4	5	3	2
		Min	16	23	31	33
		Max	29	45	40	42
		Count	15	15	15	15
Supply Air Flow Rate	(L/min)	**Mean**	**369**	**183**	**172**	**162**
		St Dev	24	1	2	1
		Min	294	182	167	160
		Max	388	185	175	164
		Count	15	15	15	15
Carbon Monoxide Concentration	(ppm)	**Mean**	0 .44	**4.6**	**13.9**	**88.4**
		St Dev	0 .01	0.2	0.2	1.4
		Min	0.43	4.1	13.7	84.6
		Max	0 .45	4.9	14.2	89.5
		Count	15	15	15	15
Carbon Dioxide Concentration	(%)	**Mean**	0.09	**0.41**	**1.20**	**2.50**
		St Dev	0.01	0.02	0.02	0.04
		Min	0.06	0.38	1.16	2.41
		Max	0.11	0.43	1.24	2.56
		Count	15	15	15	15
Oxygen Concentration	(%)	**Mean**	20.6	**17.1**	**16.1**	**15.0**
		St Dev	0.2	0.2	0.2	0.2
		Min	20.3	16.7	15.8	14.6
		Max	20.9	17.3	16.4	15.3
		Count	15	15	15	15
Static Pressure	(cm H2O)	**Mean**	**-0.02**	**-0.15**	**-0.43**	**-0.25**
		St Dev	0.02	0.05	0.08	0.13
		Min	-0.02	-0.25	-0.58	-0.53
		Max	0.05	-0.10	-0.30	-0.02
		Count	15	15	15	15

Table 2: Inhalation exposure summary data: test chemical flow rates

			Group 2 Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Carbon Monoxide Flow Rate	(mL/min)	**Mean**	**1.1**	**2.3**	**14.9**
		St Dev	0.2	0.1	0.2
		Min	0.8	2.2	14.1
		Max	1.4	2.4	15.1
		Count	15	15	15
Carbon Dioxide Flow Rate	(L/min)	**Mean**	**0.44**	**2.30**	**2.61**
		St Dev	0.02	0.07	0.06
		Min	0.41	2.20	2.50
		Max	0.49	2.40	2.70
		Count	15	15	15
Nitrogen Flow Rate	(L/min)	**Mean**	**32.7**	**44.8**	**51.6**
		St Dev	0.8	0.7	2.1
		Min	32.0	44.0	50.1
		Max	34.2	46.2	58.0
		Count	15	15	15

Table 3: Summary of estrous cycle monitoring data over five consecutive days of observation; n = (x) = (16 female rats x 5 days) minus the number of rat-days with inconclusive observations.

Estrous Cycle Phase	**Group 1** Control (80)	**Group 2** Low Dose (77)	**Group 3** Mid Dose (79)	**Group 4** High Dose (79)
Proestrus	17.5* (21.9%)	20.5* (26.6%)	19.0* (24.1%)	11.5* (14.6%)
Estrus	21.5* (26.9%)	15.0* (19.5%)	23.0* (29.1%)	24.5* (31.0%)
Metestrus + Diestrous	41.0* (51.2%)	41.5* (53.9%)	37.0* (46.8%)	43.0* (54.4%)

*** Number of rat-days observed within a specific phase of the estrous cycle**

Table 4: **(%) = Proportion of rat-days observed within a specific phase of the estrous cycle Mean organ weights in grams for adult female rats (± SD); n=16.**

Endpoint	**Group 1** Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Left Kidney	0.98 ± 0.08	0.95 ± 0.10	0.95 ± 0.10	0.95 ± 0.08
Right kidney	1.01 ± 0.07	0.97 ± 0.09	0.97 ± 0.10	0.96 ± 0.07
Spleen	0.58 ± 0.11	0.53 ± 0.09	0.56 ± 0.11	0.54 ± 0.05
Liver	7.93 ± 0.66	7.91 ± 0.83	8.00 ± 0.73	7.79 ± 0.51
Reproductive Tissues	0.72 ± 0.09	0.72 ± 0.17	0.65 ± 0.13	0.70 ± 0.13

Table 5: **Mean organ weights in grams for adult male rats (± SD); n=16; n=15 for low dose reproductive tissues.**

Endpoint	**Group 1** Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
Left Kidney	1.51 ± 0.17	1.43 ± 0.12	1.48 ± 0.16	1.58 ± 0.16
Right kidney	1.55 ± 0.15	1.45 ± 0.14	1.50 ± 0.15	1.59 ± 0.17
Spleen	0.71 ± 0.12	0.71 ± 0.06	0.68 ± 0.09	0.75 ± 0.08
Liver	11.51 ± 1.09	11.53 ± 1.08	11.45 ± 1.32	12.16 ± 1.24
Reproductive Tissues	4.27 ± 0.24	4.14 ± 0.22	4.10 ± 0.21	4.22 ± 0.34

Table 6: **Hematology values (± SD) measured in adult female rats after necropsy immediately following continuous 14-day exposure; n=(x). The standard reference ranges for CD® IGS rats female rats are indicated in endpoint column (Giknis, *et al*. 2006).**

Endpoint	**Group 1** Clean Air Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
WBC ($5.5 – 11.7 \times 10^3/\mu L$)	7.5 ± 4.0 (10)	7.4 ± 3.1 (15)	7.6 ± 4.4 (15)	9.3 ± 5.3 (15)
Lymphocytes ($3.3 – 9.5 \times 10^3/\mu L$)	4.2 ± 2.0 (10)	4.2 ± 1.5 (15)	3.8 ± 1.9 (15)	5.0 ± 2.7 (15)
% Lymphocytes	57 ± 8 (10)	59 ± 7 (15)	54 ± 9 (15)	57 ± 11 (15)
Monocytes ($0.10 – 0.90 \times 10^3/\mu L$)	0.58 ± 0.32 (10)	0.67 ± 0.38 (15)	0.70 ± 0.65 (15)	0.90 ± 0.68 (15)
% Monocytes	8 ± 2 (10)	9 ± 3 (15)	8 ± 4 (15)	9 ± 3 (15)
Neutrophils ($0.5 – 8.1 \times 10^3/\mu L$)	2.5 ± 1.6 (10)	2.3 ± 1.3 (15)	2.7 ± 1.9 (15)	3.0 ± 1.8 (15)
% Neutrophils	32 ± 5 (10)	30 ± 7 (15)	34 ± 6 (15)	31 ± 9 (15)
Eosinophils ($0.10 – 0.90 \times 10^3/\mu L$)	0.20 ± 0.25 (10)	0.13 ± 0.15 (15)	0.18 ± 0.16 (15)	0.28 ± 0.27 (15)
% Eosinophils	1.9 ± 1.9 (10)	1.6 ± 1.4 (15)	2.5 ± 1.8 (15)	2.5 ± 1.7 (15)
Basophils ($\leq 0.11 \times 10^3/\mu L$)	0.08 ± 0.13 (10)	0.05 ± 0.07 (15)	0.09 ± 0.10 (15)	0.12 ± 0.13 (15)
% Basophils	0.8 ± 1.0 (10)	0.8 ± 0.8 (15)	1.2 ± 1.1 (15)	0.9 ± 0.8 (15)
RBC ($5.9 – 8.3 \times 10^6/\mu L$)	7.3 ± 0.6 (10)	7.5 ± 1.6 (15)	6.8 ± 1.6 (15)	7.7 ± 2.1 (15)
% RDW	14.3 ± 1.0 (10)	14.4 ± 0.9 (15)	14.3 ± 0.5 (15)	15.3 ± 0.7 (15)
HB (13.5 – 15.7 g/dL)	14.4 ± 1.7 (10)	14.8 ± 2.6 (15)	13.4 ± 3.6 (15)	15.3 ± 5.1 (15)
% Hematocrit (10 – 55)	50 ± 5 (10)	52 ± 11 (15)	46 ± 11 (15)	53 ± 15 (15)
MCV ($54 – 69\ mm^3$)	68 ± 2 (10)	69 ± 3 (15)	68 ± 2 (15)	69 ± 2 (15)
MCH (18 – 22 pg)	20 ± 1 (10)	20 ± 2 (15)	20 + 1 (15)	20 ± 3 (15)
MCHC (29 – 36 g/dL)	29 ± 1 (10)	29 ± 3 (15)	29 ± 2 (15)	29 ± 5 (15)
PLT	447 ± 333	727 ± 451	683 ± 485	911 ± 495

(380 – 1210 x 10^3/µL)	(10)	(15)	(15)	(15)

Table 7: **Hematology values (± SD) measured in adult male rats after necropsy immediately following continuous 14-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, *et al.* 2006).**

Endpoint	**Group 1** Clean Air Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
WBC (3.9 – 17.2 x 10^3/µL)	8.9 ± 5.5 (14)	9.5 ± 3.8 (14)	7.6 ± 3.9 (15)	10.3 ± 3.3 (15)
Lymphocytes (1.2 – 10.7 x 10^3/µL)	4.9 ± 3.8 (14)	5.0 ± 2.0 (14)	3.6 ± 1.7 (15)	5.0 ± 1.7 (15)
% Lymphocytes	55 ± 14 (14)	55 ± 11 (14)	49 ± 9 (15)	50 ± 8 (15)
Monocytes (0.10 – 0.90 x 10^3/µL)	0.71 ± 0.50 (14)	0.95 ± 0.62 (14)	0.75 ± 0.48 (15)	1.14 ± 0.61 (15)
% Monocytes	8 ± 3 (14)	10 ± 4 (14)	10 ± 3 (15)	11 ± 5 (15)
Neutrophils (0.5 – 8.1 x 10^3/µL)	3.2 ± 1.9 (14)	3.4 ± 1.7 (14)	3.2 ± 1.9 (15)	3.9 ± 1.3 (15)
% Neutrophils	35 ± 13 (14)	35 ± 8 (14)	39 ± 8 (15)	38 ± 6 (15)
Eosinophils (0.10 – 0.40 x 10^3/µL)	0.12 ± 0.13 (14)	0.07 ± 0.08 (14)	0.08 ± 0.09 (15)	0.15 ± 0.16 (15)
% Eosinophils	1.2 ± 1.0 (14)	0.6 ± 0.7 (14)	1.2 ± 1.1 (15)	1.3 ± 1.1 (15)
Basophils (≤ 0.20 x 10^3/µL)	0.05 ± 0.06 (14)	0.02 ± 0.04 (14)	0.05 ± 0.06 (15)	0.05 ± 0.08 (15)
% Basophils	0.6 ± 0.5 (14)	0.2 ± 0.3 (14)	0.7 ± 0.8 (15)	0.4 ± 0.5 (15)
RBC (5.1 – 9.0 x 10^6/µL)	6.8 ± 1.5 (14)	7.1 ± 1.0 (14)	7.3 ± 1.6 (15)	7.6 ± 1.3 (15)
% RDW	15.7 ± 0.9 (10)	15.7 ± 0.6 (15)	15.7 ± 1.0 (15)	16.6 ± 0.8 (15)
HB (8.6 – 16.3 x 10^6/µL)	14.0 ± 3.0 (14)	14.6 ± 2.8 (14)	14.7 ± 3.2 (15)	16.6 ± 3.3 (15)
% Hematocrit (28 – 55)	48 ± 11 (14)	50 ± 9 (14)	51 ± 11 (15)	55 ± 9 (15)
MCV (51 – 73 mm^3)	70 ± 2 (14)	70 ± 5 (14)	70 ± 3 (15)	72 ± 3 (15)
MCH (18 – 21 pg)	21 ± 3 (14)	20 ± 2 (14)	20 ± 2 (15)	22 ± 1 (15)
MCHC (29 – 37 g/dL)	30 ± 3 (14)	29 ± 1 (14)	29 ± 2 (15)	30 ± 2 (15)

PLT (380 – 1210 x 10^3/µL)	390 ± 288 (10)	820 ± 477 (15)	736 ± 424 (15)	1023 ± 364 (15)

Table 8: **Serum chemistries (± SD) measured in adult female rats after necropsy immediately following continuous 14-day exposure; n=(x). The standard reference ranges for CD® IGS female rats are indicated in endpoint column (Giknis, *et al.* 2006).**

Endpoint	**Group 1** Clean Air Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
TP (5.7 – 8.9 g/dL)	7.18 ± 0.77 (13)	6.96 ± 0.39 (16)	6.87 ± 0.47 (15)	7.13 ± 0.49 (15)
ALB (3.3 – 6.7 g/dL)	3.83 ± 0.44 (13)	3.96 ± 0.30 (16)	3.87 ± 0.34 (16)	4.15 ± 0.35 (15)
ALKP (90 – 205 U/L)	115 ± 32 (13)	139 ± 45 (16)	146 ± 52 (16)	116 ± 40 (15)
ALT (23 – 186 U/L)	101 ± 55 (13)	79 ± 27 (16)	73 ± 20 (16)	76 ± 37 (15)
AST (78 – 226 U/L)	187 ± 92 (12)	136 ± 60 (15)	119 ± 45 (16)	126 ± 68 (15)
BUN (10 – 25 mg/dL)	19.2 ± 4.8 (13)	18.5 ± 1.7 (15)	19.8 ± 2.9 (16)	21.1 ± 2.8 (16)
CHOL (47 – 92 mg/dL)	49.4 ± 14.0 (13)	52.3 ± 13.2 (16)	51.8 ± 12.0 (16)	51.0 ± 15.2 (16)
CK (117 – 531 U/L)	422 ± 223 (12)	234 ± 109 (12)	276 ± 163 (15)	254 ± 129 (16)
CREA (0.50 – 0.90 mg/dL)	0.63 ± 0.15 (13)	0.66 ± 0.07 (16)	0.65 ± 0.07 (16)	0.68 ± 0.10 (16)
GLU (81 – 185 mg/dL)	127 ± 15 (13)	148 ± 37 (15)	150 ± 30 (15)	165 ± 26 (16)
TBIL (0.10 – 1.00 mg/dL)	0.34 ± 0.31 (13)	0.21 ± 0.18 (15)	0.27 ± 0.13 (15)	0.29 ± 0.18 (13)
TRIG (30 – 205 mg/dL)	41 ± 7 (13)	60 ± 19 (15)	53 ± 10 (14)	81 ± 48 (16)
Na+ (140 – 156 mEq/L)	160 ± 17 (12)	152 ± 3 (16)	153 ± 2 (16)	154 ± 8 (16)
K+ (4.1 – 6.9 mEq/L)	5.81 ± 0.87 (13)	6.68 ± 0.65 (14)	6.85 ± 0.72 (15)	6.83 ± 1.03 (15)
Cl- (95 – 111 mEq/L)	106 ± 7 (12)	104 ± 1 (16)	105 ± 2 (16)	105 ± 4 (16)

Table 9: **Serum chemistries (± SD) measured in adult male rats after necropsy immediately following continuous 14-day exposure; n=(x). The standard reference ranges for CD® IGS male rats are indicated in endpoint column (Giknis, *et al.* 2006).**

Endpoint	**Group 1** Clean Air Control	**Group 2** Low Dose	**Group 3** Mid Dose	**Group 4** High Dose
TP (5.6 – 8.1 g/dL)	6.36 ± 0.60 (13)	6.45 ± 0.31 (15)	6.63 ± 0.41 (15)	6.52 ± 0.32 (16)
ALB (3.2 – 5.2 g/dL)	3.27 ± 0.34 (13)	3.47 ± 0.21 (15)	3.55 ± 0.26 (15)	3.51 ± 0.17 (16)
ALKP (136 – 268 U/L)	228 ± 69 (13)	251 ± 60 (15)	244 ± 65 (15)	292 ± 43 (16)
ALT (27 – 97 U/L)	74 ± 14 (12)	65 ± 8 (14)	70 ± 10 (15)	80 ± 16 (16)
AST (77 – 246 U/L)	170 ± 90 (12)	114 ± 37 (14)	135 ± 57 (14)	143 ± 49 (16)
BUN (10 – 22 mg/dL)	15.2 ± 2.2 (13)	15.3 ± 2.2 (15)	16.4 ± 2.9 (15)	16.2 ± 2.7 (16)
CHOL (24 – 92 mg/dL)	33.9 ± 8.6 (13)	38.0 ± 9.2 (15)	37.9 ± 10.3 (15)	27.4 ± 11.2 (16)
CK (56 – 477 U/L)	352 ± 175 (10)	214 ± 88 (13)	268 ± 159 (12)	222 ± 117 (15)
CREA (0.40 – 0.80 mg/dL)	0.55 ± 0.15 (13)	0.66 ± 0.10 (15)	0.68 ± 0.07 (15)	0.71 ± 0.11 (16)
GLU (85 – 197 mg/dL)	123 ± 14 (13)	139 ± 21 (15)	134 ± 23 (15)	165 ± 59 (16)
TBIL (0.10 – 1.00 mg/dL)	0.20 ± 0.13 (11)	0.19 ± 0.15 (15)	0.24 ± 0.20 (14)	0.15 ± 0.09 (16)
TRIG (46 – 208 mg/dL)	46 ± 10 (13)	62 ±23 (15)	56 ± 13 (15)	47 ± 14 (16)
Na+ (141 – 157 mEq/L)	159 ± 13 (13)	154 ± 3 (15)	154 ± 2 (15)	155 ± 5 (16)
K+ (4.7 – 7.3 mEq/L)	6.02 ± 0.52 (11)	6.51 ± 0.75 (14)	6.69 ± 0.78 (15)	6.68 ± 0.85 (16)
Cl- (97 – 110 mEq/L)	106 ± 4 (12)	104 ± 2 (15)	104 ± 1 (15)	103 ± 2 (16)

Table 10: Histopathological lesions identified in female animals.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Animals Examined	n=16	n=16	n=16	n=16
Heart				
Chronic Infiltrates	2 (2+)	0	0	0
Pancreas				
Chronic Infiltrates	0	0	0	5 (5+)
Degenerative Changes	0	0	0	5 (5+)
Liver				
Chronic Infiltrates	7 (7+)	14 (14+)	16 (14+/1++/1+++)	16 (15+/1++)
Degenerative Changes	1 (1+)	0	0	2 (2+)
Spleen				
Chronic Infiltrates	0	0	1 (1++)	0
Degenerative Changes	0	0	1 (1++)	0
Necrosis	0	0	1 (1++)	0
Kidneys				
Chronic Infiltrates	4 (4+)	3 (3+)	6 (6+)	8 (6+/2++)
Progressive Nephropathy	9 (9+)	8 (8+)	9 (9+)	10 (8+/2++)
Pituitary Gland				
Pseudocyst	1 (1+)	0	0	0

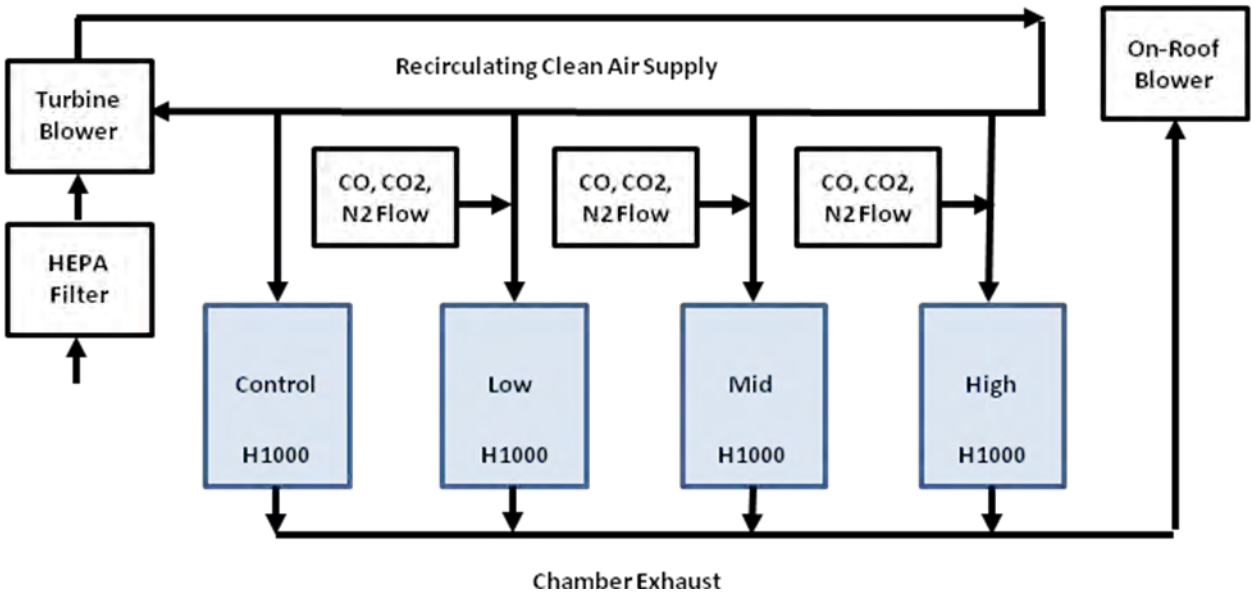
Severity: **Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++**
Incidence: **(in parentheses)**

Table 11: Histopathological lesions identified in male animals.

Endpoint	Group 1 Control	Group 2 Low Dose	Group 3 Mid Dose	Group 4 High Dose
Animals Examined	n=16	n=16	n=16	n=16
Heart				
Chronic Infiltrates	1 (1+)	0	0	0
Cardiomyopathy	0	1 (1+)	0	0
Pancreas				
Chronic Infiltrates	0	0	3 (3+)	4 (3+/1++)
Degenerative Changes	0	0	3 (3+)	4 (3+/1++)
Liver				
Chronic Infiltrates	9 (9+)	12 (12+)	14 (13+/1++)	15 (14+/1++)
Degenerative Changes	0	1 (1+)	0	0
Spleen				
Degenerative Changes	0	0	1 (1+)	0
Necrosis	0	0	1 (1+)	0
Kidneys				
Chronic Infiltrates	9 (9+)	5 (5+)	6 (5+/1++)	12 (12+)
Progressive Nephropathy	10 (10+)	9 (9+)	13 (12+/1++)	14 (14+)
Pituitary Gland				
Pseudocyst	1 (1+)	0	0	0
Male Reproductive Organs				
Prostatitis	0	1 (1++)	0	6 (1+/5++)
Sperm Granuloma (Epididymis)	0	0	0	1 (1++)
Degenerative Changes (Testicular Seminiferous Tubule)	1 (1+)	1 (1+)	0	0

Severity: **Minimal to Rare = +; Mild = ++; Moderate = +++; Marked to Severe = ++++**
Incidence: **(in parentheses)**

Figure 1 **Diagrammatic representation of the exposure system**



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13. SUPPLEMENTARY NOTES

14. ABSTRACT
Recent congressional approval allowing women to serve in the submarine service necessitates additional investigation into the suitability of existing submarine breathing air standards for women. This study evaluates the general, reproductive and developmental health effects upon male and female rats exposed to mixtures of three critical submarine atmospheric components (CO, CO_2, and O_2) at concentrations that represent current submarine. The study is divided into three consecutive phases including a range finding phase, a 28-day exposure phase with reproductive assessment and a 90-day 2-generation reproductive/developmental evaluation. This technical report presents the findings of the first phase of the study that included controls and three different mixtures of CO, CO_2 and low O_2. The exposure concentrations at all doses were well tolerated by the rats and will be used in the subsequent phases. Pathological findings were unremarkable, or incidental to exposure, and blood analysis results were within normal clinical parameters. There were no identified effects to reproductive tissues from exposure to the mixtures. Due to the short duration of the exposures in this range-finding phase of the study, no definitive conclusions should be drawn at this time regarding the toxicities, or lack thereof, of these mixtures.

15. SUBJECT TERMS
Inhalation, carbon monoxide, carbon dioxide, hypoxia, reproductive toxicity

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